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USE OF MULTIVARIATE ANALYSIS IN MINERAL ACCUMULATION OF ROCKET (*ERUCA SATIVA*) ACCESSIONS

M. KADRI BOZOKALFA[1], Dursun EŞIYOK[1], Bülent YAĞMUR[2]

[1]Department of Horticulture, Faculty of Agriculture, Ege University, İzmir, Turkey
[2]Department of Soil Science, Faculty of Agriculture, Ege University, İzmir, Turkey

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The leafy vegetables contain high amount of mineral elements and health promoting compound. To solve nutritional problems in diet and reduced malnutrition among human population selection of specific cultivar among species would be help increasing elemental delivery in the human diet. While rocket plant observes several nutritional compounds no significant efforts have been made for genetic diversity for mineral composition of rocket plant accessions using multivariate analyses technique. The objective of this work was to evaluate variability for mineral accumulation of rocket accessions revealed by multivariate analysis to use further breeding program for achieve improving cultivar in targeting high nutrient concentration. A total twelve mineral element and twenty-three *E. sativa* accessions were investigated and considerable variation were observed in the most of concentration the principal component analysis

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Corresponding author: M. Kadri Bozokalfa, Department of Horticulture, Faculty of Agriculture, Ege University, Bornova 35100 İzmir, Turkey,Tel & Fax: +90 232 3881865, e-mail: mehmet.kadri.bozokalfa@ege.edu.tr

explained that 77.67% of total variation accounted for four PC axis. Rocket accessions were classifies into three groups and present outcomes of experiments revealed that the first three principal components were highly valid to classify the examined accessions and separating mineral accumulations. Significant differences exhibited in mineral concentration among examined rocket accessions and the result could allow selecting those genotypes with higher elements.

Key words: diversity, mineral accumulation, multivariate analysis rocket plant

INTRODUCTION

Green leafy vegetable *E. sativa* is member of Brassicaeae family and originated from Mediterranean countries. Rocket is much appreciated vegetable in Europe, North America, Argentina and South Africa, and also it has been cultivated in Central and Western Asia for seed oil (YANIV *et al.,* 1998). The linseed oil which is produce from rocket and *Linum usitatissimum* seeds started thousands year ago in Central Anatolia (ERTUG, 2000) for use in lambs, to lubricate wooden-wheeled carts and in the processing of water-buffalo skins. The rocket plants are use in several ways as condiment in salads, spice, cooked vegetables and functional plants (KIM *et al.,* 2006).

There is increasing interest for potential nutritional composition of vegetables effect on human health and the role in metabolic function in diet. Thus, researchers have been interested in improving the nutritional quality of plants, with respect to both nutrient composition and concentration (BOUIS, 1996). In this respects Brassicae family is substantial role in human diet and represents high amount of vitamin C, carotenes, phenolic compound and health promoting compound such as glucosinolates (D'ANTUONO *et al.,* 2008). In addition Brassicae family rich sources of bioactive compounds which has chemoprotective properties such as glucosinolates, flavonoids, fiber, folate and carotenoids (JIN *et al.,* 2009). Although rocket plant is not considerable significant economic value comparing to the other Brassicae vegetables, consumption and growing areas is increase due to containing high mineral elements (EŞIYOK *et al.,* 2010), observed potential health promoting compound, and it's easy to grown year around with low cost value. The rocket plant is also containing high amount of vitamin C, glucosinolates, flavonoids and phenolic compound (MARTINEZ-SANCHEZ *et al.,* 2006) and also rich in mineral elements (KAWASHIMA and VALENTE-SOARES 2003).

In today devoting more attention to flavor and nutritional quality of fruits and vegetables is strongly recommended (KADER, 2008), people are not only looking for taste, aroma and visual properties of edible parts but also seeking health benefits of fresh fruits and vegetables (BORAH *et al.,* 2010), and eventually consumer oriented quality traits is becoming increasingly important for plant breeders. To solve nutritional problems in human diet and reduced malnutrition among population selection specific cultivar among species would be help increasing elemental delivery in the human diet (KOPSELL *et al.,* 2004). Therefore it is very important to identify germplasm with high levels of the micronutrients in selection program to enhance the nutritional composition of species through the conventional plant breeding. Genetic diversity is imply the variability of species and can improve quality of cultivated plants (MILOSEVIC *et al.,* 2010). Thus, it is important to assessment germplasm with high mineral concentration and application multivariate methods of analysis could be useful tool to selecting genotype (SANTOS *et al.,* 2010) among plant genetic resources which is a good source for comprehensive breeding program.

The present research was undertaken to assess the degree of diversity in the mineral accumulation of genetically diverse rocket plant population using multivariate analyses.

MATERIALS AND METHODS

The study was conducted at the Ege University, Faculty of Agriculture, Department of Horticulture in Bornova, Izmir, Turkey. To determine genetic variation of mineral accumulation 23 accessions of rocket which 20 had been obtained from the USDA-GRIN (originating from several regions of the world), one sample of *E. sativa*, gathered from a market in the East of Turkey, and one *E. sativa* Mill. cultivar from Italy (Table 1). Seeds were sown in a 20 liter volume pots, containing a mixture of peat and perlite (3:1 v/v) as substrate and randomized complete block design were applied with three replications. A total 30 seeds were sown in each pot and trimmed after true leaves expansion and allowing 10 plants per pot. No chemical fertilizer, fungicide and insecticide were applied during cultivation, and weeds were controlled mechanically by hand until the plant reached harvest maturity. Plant samples were harvested by hand at leaves fully matured and ready for edible use.

In the experiment each accession leaf samples in analyzed in triplicate and 12 traits were recorded (NO_2, NO_3, N, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn). The total amounts of NO_3, NO_2 and N in the leaf samples were determined by modified Kjeldahl method, P in wet digested samples with colorimetry, potassium K, Ca and Na, with flame photometry, Mg, Fe, Zn, Cu and Mn using atomic absorption spectrometry.

Multivariate analysis techniques were applied in collected data and principal component analysis (PCA) was carried out for quantitative mineral concentration, and the total of variation explained was calculated as the sum of extracted Eigen-values. The PCA techniques can be used to determine the variables containing the maximum possible variance and to reduce the information of a multidimensional data set in that it can be displayed in a scatter plot with only three axes. Hierarchical agglomerative clustering was then performed on the principle component axes obtained, using the Ward criterion (SNEATH and SOKAL, 1973). SPSS program (SPSS 16.0)

Table 1. List of genotypes used for determination of mineral composition

Species (botanical name)[a]	Donor[b]	Accession number/ genotype name/	Collection locale/country of origin
Eruca sativa	FRC	*Eruca sativa- I*	Local cultivar- Italy
Eruca sativa Mill.	LC	*Eruca sativa - II*	Local cultivar- Turkey
Eruca sativa	LF	*Eruca sativa*	Konya-Turkey
Eruca sativa	USDA-ARS	PI 170362	Turkey
Eruca sativa	USDA-ARS	PI 173902	Turkey
Eruca sativa	USDA-ARS	PI 175720	Turkey
Eruca sativa	USDA-ARS	PI 178901	Turkey
Eruca sativa	USDA-ARS	PI 179279	Turkey
Eruca sativa	USDA-ARS	PI 183233	Egypt
Eruca sativa	USDA-ARS	PI 217829	Pakistan
Eruca sativa	USDA-ARS	PI 251490	Iran
Eruca sativa	USDA-ARS	PI 255664	Afghanistan
Eruca sativa	USDA-ARS	PI 261629	Spain
Eruca sativa	USDA-ARS	PI 311742	Poland
Eruca sativa	USDA-ARS	PI 344365	Turkey
Eruca sativa	USDA-ARS	PI 390143	Pakistan
Eruca sativa	USDA-ARS	PI 407630	Turkey
Eruca sativa	USD-ARS	PI 426198	Afghanistan
Eruca sativa	USDA-ARS	PI 432339	Cyprus
Eruca sativa	USDA-ARS	PI 603033	Pakistan
Eruca sativa	USDA-ARS	PI 633202	England
Eruca sativa	USDA-ARS	PI 597835	Algeria
Eruca sativa	USDA-ARS	PI 633210	China

[a] According to ARS-GRIN sample seed package information

[b]EUFA-RC: Ege University Faculty of Agriculture, Turkey researchers collected, ARS-GRIN: United States Department of Agriculture Agricultural Research Service North Central Regional Plant Introduction Station, Ames, USA, FRC: Received from foreign researchers Jules Janick collected in Italy, LF: Local farmer supported, LC: Local cultivar sales in market.

RESULTS AND DISCUSSION

A total twenty three accession was evaluated based on mineral accumulating in leaves and there were highly significant differences in all the mineral composition among the examined accessions. Many accession observed large amount of important mineral element for human nutrition particularly in K, Mn, Zn, and Cu concentrations. In order to evaluate mineral accumulation among *E. sativa* accessions in a diversity context principal component analysis were apply by all the twelve variables. The principal component analysis accounted for more than 77.67% of total variation encountered and four PC axes extracted in total variation, and the first three components was 64.24% in examined plant populations (Table 2). The first PC accounting for 28.61% of the variation comprises Na, NO_2, NO_3, Fe and Ca. The second component emphasized N and Mg and accounted for 19.08% of the variation. The third component accounted for 16.55% of the total variation and concerned with Zn, Mn and P. The fourth component constituted 13.43% of the variation and Cu and K are characters contribute to fourth axes.

Table 2. Eigen values proportion of variability and minerals contributed to the first four PC's of E. sativa accessions.

	PC axis			
	1	2	3	4
Eigen values	3.43	2.29	1.99	1.61
Explained proportion of variation (%)	28.61	19.08	16.55	13.43
Cumulative proportion of variation (%)	28.61	47.69	64.24	77.67
Mineral Elements	Eigen vectors			
Na	0.924	-0.072	-0.240	0.071
NO_2	-0.864	-0.016	0.059	0.211
NO_3	-0.816	-0.040	0.080	0.234
Fe	0.736	-0.025	0.314	-0.315
Ca	0.695	-0.259	-0.168	0.388
N	0.003	0.966	0.004	-0.104
Mg	-0.121	0.963	0.009	-0.013
Zn	-0.047	-0.301	0.800	0.107
Mn	-0.099	0.092	0.780	-0.112
P	-0.060	0.359	0.583	0.222
Cu	-0.103	0.110	0.374	0.827
K	0.317	0.338	0.252	-0.699

A hierarchical agglomerative procedure was constructed to determine the relationships among accessions. The dendrogram of 23 *E. sativa* accessions over 12 mineral elements revealed that examined collection divided into three cluster basis on mineral accumulation (Figure 1). The mean values of accessions in each clusters are given table 3, first cluster comprises nine accessions and showed the highest NO_2 (323.18 mg kg^{-1}), Ca (0.837 mg g^{-1}), and Cu (8.842 mg kg^{-1}), whilst lower P (0.477 mg g^{-1}), K (5.085 mg g^{-1}), Fe (103.34 mg kg^{-1}), Zn (40.512 mg kg^{-1}) and Mn (40.366 mg kg^{-1}) concentration. The second cluster consist of seven accessions and the highest Na (0.668 mg g^{-1}), Fe (129.83 mg kg^{-1}), Zn (48.198 mg kg^{-1}), Mn (51.52 mg g^{-1}) and while the lowest NO_3 (315.91 mg kg^{-1}), NO_2 (0.029 mg kg^{-1}), N (2.222 mg g^{-1}), Mg (2.427 mg g^{-1}). The highest N (2.580 mg g^{-1}), P (0.490 mg g^{-1}), K (6.023 mg g^{-1}), Mg (2.427 mg g^{-1}) obtained from third cluster and seven accessions was placed. The high total variance explained by the first three axes which observed 64.24% of the total variation and the major part of variance of the data set comes to lie on the first, second and third axes. In order to demonstrate relationships of investigated minerals the first three principal co-ordinates are given 3D (dimension) scatter plot (Figure 2). Mineral elements separate their corresponding value of explained proportion of variation in the scatter plot.

Table 3. Mean values for five clusters based on twelve mineral elements

Mineral elements	Cluster I	Cluster II	Cluster III
NO_3 (mg kg^{-1})	323.18	315.91	320.59
NO_2 (mg kg^{-1})	0.030	0.029	0.030
N (mg g^{-1})	2.423	2.222	2.580
P (mg g^{-1})	0.477	0.483	0.490
K (mg g^{-1})	5.085	5.450	6.023
Ca (mg g^{-1})	0.837	0.842	0.744
Mg (mg g^{-1})	2.311	2.092	2.427
Na (mg g^{-1})	0.642	0.668	0.581
Fe (mg kg^{-1})	103.34	129.83	112.56
Cu (mg kg^{-1})	8.842	7.763	6.173
Zn (mg kg^{-1})	40.512	48.198	42.903
Mn (mg kg^{-1})	40.366	51.523	50.531

Vegetables and fruits are excellent source for mineral, vitamins, phenolic compound and dietary fiber. Among vegetables and herbs the importance of underutilized crops such as wild and cultivated rocket plant increase due to containing large amount of required nutrient in human diet. The rocket plant is cultivated in several countries and consumption is continued to increase due to containing large amount mineral element and health promoting compound. In order to reduce the mineral deficiencies and malnutrition in human diet enhancing consumption of nutritionally improved species and cultivar. Rocket has substantial vegetables rich in potentially health promoting compound and accumulate large amount of mineral elements as a green salad, besides health benefits it is available all over the year and grown easily (AHMED *et al.,* 2000). The assessment of genetic variation among plant population is widely apply in several plant species but limited information is available mineral concentration levels in vegetables and have achieved only little concern in diversity context. Moreover multivariate analysis was not applied to assessment diversity context in the mineral concentration among rocket germplasm, yet.

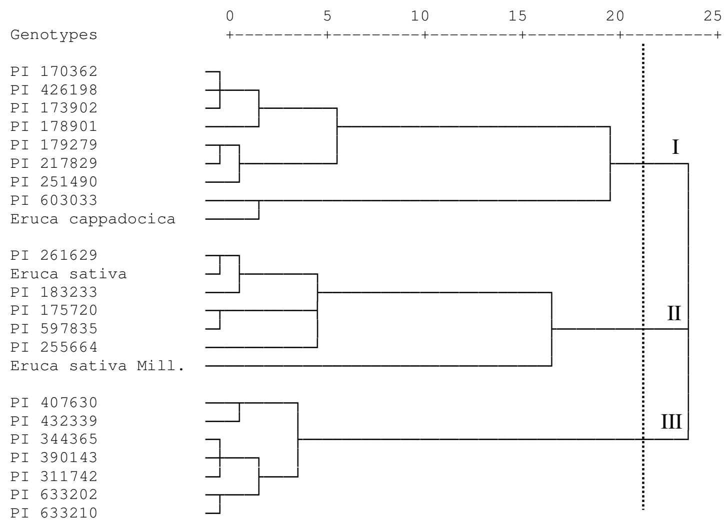

Figure 1. Genetic relationship among 23 accessions of *E. sativa* based on mineral elements by Ward's clustering method.

Principal component analysis and hierarchical clustering pattern which is comprises multivariate analysis has been apply in order to evaluate characterize and classify germplasm based on mineral composition in several species. This study focused on evaluating rocket germplasm mineral concentration by multivariate techniques to bring into the open information on differentiation among population for further breeding programme. In the present paper showed that significant differences in mineral concentration among examined rocket plant collections. The results of experiments revealed that the first three principal components were highly valid to classify the examined accessions and separating mineral accumulations. Mineral concentration, accumulation, and nutritional composition in edible of parts vegetables such as onion (*Allium cepa* L.) (CHOPE and TERRY 2009), kale (*Brassica oleracea* L. var. *acephala* DC.) (FADIGAS *et al.,* 2010), common bean (*Phaseolus vulgaris* L.) (SANTOS *et al.,* 2010) were investigated in diversity context and showed a wide variability among investigated genotypes. In addition ANDRE *et al.,* (2007) showed a wide variability in micronutrient levels among the genetically diverse potato (*Solanum tuberosum* L.) core collection and indicate genetic differences in the control of these components.

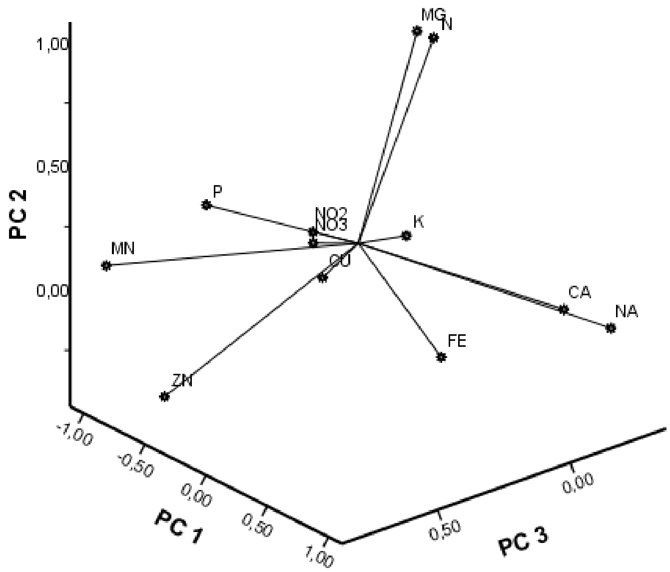

Figure 2. Patterns of relationships among *E. sativa* accession due to the first three principal co-ordinates.

DIZUDA and PITURA (2008) reported that nutritional value, chemical composition of plant tissue may greatly vary depending on the cultivation, fertilization. While soil mineral concentration, availability, fertilization and environment may influenced on nutrient accumulation in plant tissue, genetic variability is considerable influenced on mineral composition of plant. In present investigation plants were grown in same growing condition and same cultivation practices were apply in all pots and great variability was determined among accessions. The parts of variation could be explained by genotypic variation which is underlined among wild and cultivated rocket plant germplasm (BOZOKALFA *et al.,* 2009). Mineral concentration differences among *Brassica rapa* L. accessions was also underlined by WU *et al.,* (2007) and explained with genetic variation. Furthermore genetic variation in mineral accumulation in edible part of onion, kale, turnip, common bean, rice, and cabbage was assessed by several researchers (CHOPE and TERRY, 2009; FADIGAS *et al.,* 2010; SANTOS *et al.,* 2010; GREGORIO *et al.,* 1999; HARRISON and BERGMANN (1981). Moreover BUKHSH *et al.,* (2007) underlined that nutrient element compositions may vary due to botanical structures of plant. JIN *et al.,* (2009) reported that plant growth condition and postharvest storage conditions have an impact on the range and quantity of phytonutrients accumulated and variability indicated among genotype.

As a conclusion plant breeders are taking into account consumer preferences, today most of the breeding program is focus on to reduce malnutrition or mineral deficiencies in developing countries by improving mineral concentration of edible part of vegetables. Increasing the contents of nutrition in plant food through plant breeding was proposed as a strategy for combination widespread mineral deficiencies. Therefore identification nutritionally rich germplasm could be enhancing the success of breeding program. Genotypic selection may reduce dietary micronutrient deficiencies (WELCH *et al.,* 2000) and also selection specific cultivar among species would be help increasing elemental delivery in the human diet (KOPSELL *et al.,* 2004). The rocket plant contains various health benefit compounds such as thiocyanates and isothiocyanates, in addition due to their glucosinolates composition the plant could be classifies as a functional foods (SLATER and MANVILLE 1993; MILAN *et. al.,* 1996). Furthermore it has a potential to become oil crop in Turkey (BOZOKALFA *et al.,* 2010). The present research underlined that rocket plant accumulate large amount of mineral element and exhibited great variability which could be explanation by genotypic variation among accessions. Having this in mind and banking on available expertise examined germplasm are good source for future breeding programme either for mineral concentration and nutritive value.

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ANALIZA AKUMULACIJE MINERALA U RAZLIČITIM GENOTIPOVIMA *ERUCA SATIVA*

M. KADRI BOZOKALFA[1], Dursun EŞIYOK[1] , Bülent YAĞMUR[2]

[1] Odelenje za Hortikulturu, Poljoprivredni fakultet Ege Univerziteta, Izmir, Turska
[2] Odelenje za izučavanje zemljišta, Poljoprivredni fakultet Ege Univerziteta, Izmir, Turska

I z v o d

Lisnato povrće, uključujići i kupusnjače sadrži veliku količinu mineralnih elemenata i komponenata značajnih za zdravlje. Rešenju problema dijete i nepravilne ishrane ljudske populacije pomogla bi selekcija genotipova specifičnog kvaliteta unutar biljne vrste. Iako kupusnjače sadrže nekoliko hranljivih elemenata nisu vršena istraživanja genetičke divergentontnosti mineralnog sastava lišća primenom metoda analize multiple variance. Predmet ovih istraživanja je utvrđivanje varijabilnosti akumulacije minerala u različitim genotipovima analizom varijanse u cilju oplemenjivanja na visok sadržaj hranljivih elemenata. Vršena su ispitivanja sadržaja dvanaest minerala u dvadeset tri genotipa *Eruca sativa*. Utvrđene su statistički značajne razlike između ispitivanih genotipova. Genotipovi su klasifikovani u tri grupe i rezultati analize multiple varijanse su pokazali da su prve tri glavne komponente variranja visoko značajne za klasifikaciju ispitivanih genotipova prema akumulaciji minerala. Značajne razlike utvrđene između genotipova u koncentraciji minerala omogućuju odabiranje onih sa visokim sadržajem ispitivanih mineralnih elementa.

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